

WHAT IS CLAIMED IS:

1. A test medium for detecting, identifying and qualifying or quantifying first and second biological entities, said test medium comprising:

a nutrient base medium including ions of a salt; and

a chromogenic substrate and a nonchromogenic substrate;

wherein, the first biological entity is responsive to said nonchromogenic substrate and the second biological entity is responsive to said chromogenic substrate;

whereby, aggregations of the first biological entity present in said test medium are substantially black and aggregations of the second biological entity present in the test medium are a second color, said second color being distinguishable from said substantially black.

2. The test medium of claim 1, further comprising an antibiotic to inhibit growth of aggregations of a third biological entity present in said test medium.

3. The test medium of claim 2, wherein said antibiotic comprises nalidixic acid.

4. The test medium of claim 1, wherein the first biological entity is responsive to said chromogenic substrate, whereby the aggregations of the first biological entity are the substantially black.

5. A test medium for detecting, identifying and qualifying or quantifying first, second and third biological entities, said test medium comprising:

a nutrient base medium including ions of a salt;

first and second chromogenic substrates; and

a nonchromogenic substrate;

wherein, the first and second biological entities are responsive to said first and said second chromogenic substrates, respectively, and the third biological entity is responsive to said nonchromogenic substrate;

whereby, aggregations of the first biological entity present in said test medium are a first color, aggregations of the second biological entity present in the test medium are a second color, and aggregations of the third biological entity present in the test medium are substantially black.

6. The test medium of claim 5, wherein the third biological entity is responsive to said first chromogenic substrate, whereby the aggregations of

the third biological entity are substantially black.

7. The test medium of claim 6, wherein the third biological entity is responsive to said second chromogenic substrate, whereby the aggregations of the third biological entity are substantially black.

8. The test medium of claim 5, wherein the second biological entity is responsive to said first chromogenic substrate, whereby said second color is formed from said first and said second chromogenic substrates.

9. A test medium for detecting, quantifying, qualifying, identifying and differentiating general coliforms and *E. coli* under ambient light, said test medium comprising:

a nutrient base medium including ions from a salt;

a first substrate capable of forming a first water insoluble component of a first color in the presence of *E. coli* and said ions, said first color being substantially black; and

a second substrate capable of forming a second water insoluble component of a second color in the presence of general coliforms, said second color being distinguishable from said first color;

whereby, colonies of *E. coli* present in said test medium are indicated by said first substantially black color and colonies of general coliforms are indicated by said second color.

10. The test medium of claim 9, wherein said first substrate consists essentially of a  $\beta$ -D-glucuronide.

11. The test medium of claim 9, wherein said salt comprises ferric ammonium citrate and said ions comprise ferric ions.

12. The test medium of claim 9, wherein said second substrate consists essentially of a  $\beta$ -D-galactoside.

13. The test medium of claim 12, wherein said first substrate consists essentially of a  $\beta$ -D-glucuronide.

14. The test medium of claim 9, further including an inhibitor to prevent the growth of *Aeromonas* in said test medium.

15. The test medium of claim 14, wherein said inhibitor comprises nalidixic acid.

16. The test medium of claim 9, further comprising a third substrate capable of forming a third water insoluble component of a third color in the presence of one of *Salmonella* and *Shigella spp.*, said third color being distinguishable from said first and said second colors, whereby said test medium is capable of detecting, quantifying and differentiating *E. coli*, general coliforms and the one of the *Salmonella* and *Shigella*.

17. The test medium of claim 16, wherein said third substrate is capable of forming said third water insoluble component of said third color in the presence of general coliforms present in said test medium, whereby general coliforms are indicated in said test medium as a fourth color, said fourth color being a combination of said second color and said third color, said fourth color being visually distinguishable from said first and said third colors.

18. The test medium of claim 17, wherein said second substrate consists essentially of a  $\beta$ -D-galactoside.

19. The test medium of claim 18, wherein said third substrate consists essentially of an  $\alpha$ -galactoside.

20. A test medium for detecting, quantifying and differentiating biological entities under ambient light, said test medium comprising:

a nutrient base medium including ions of a salt;

a first substrate capable of forming a first water insoluble component of a first color in the presence of a first type of biological entity and metallic ions of said metallic salt, said first color being substantially black;

a second substrate capable of forming a second water insoluble component of a second color in the presence of a second type of biological entity, said second color being distinguishable from said first color; and

an inhibitor, whereby colonies of an additional biological entity are substantially prevented from growing in said medium.

21. The test medium of claim 20, wherein said inhibitor comprises nalidixic acid.

22. A method for detecting, quantifying, qualifying and differentiating under ambient light general coliforms, *E. coli*, and at least one of the genera *Aeromonas*, *Salmonella* and *Shigella* in a test sample, said method comprising:

5 providing a nutrient base medium including first, second and third  
substrates, each of the substrates being capable of forming a water insoluble component in  
the presence of at least one of general coliforms, *E. coli*, *Aeromonas*, *Salmonella* and  
*Shigella*;

10 selecting the substrates such that colonies of *E. coli* produced in the test  
medium are a first color, colonies of general coliforms produced in the test medium are a  
second color, and colonies of at least one of *Aeromonas* and *Salmonella* and *Shigella*  
produced in the test medium are a third color, each of the colors being visually  
distinguishable;

inoculating the test medium with the test sample;

incubating the test medium; and

15 examining the test medium for the presence of first colonies having the first  
color, second colonies having the second color and third colonies having the third color,  
the first colonies being *E. coli*, the second colonies being general coliforms, and the third  
colonies being at least one of *Aeromonas*, and *Salmonella* and *Shigella*.

23. The method of claim 22, further comprising adding a salt to the test  
medium to react with one of the substrates, whereby one the colors is substantially black.

24. The method of claim 22, further comprising examining the test medium for  
the presence of fourth colonies having a fourth color, wherein the substrates are selected  
such that colonies of *Aeromonas* are the third color and colonies of one of *Salmonella* and  
*Shigella* are the fourth color, the fourth color being visually distinguishable from the first,  
the second and the third colors.

25. The method of claim 24, wherein the substrates are selected such that the  
fourth color is substantially teal-green.

26. The method of claim 22, wherein the substrates are selected such that the  
first color is substantially black.

27. The method of claim 26, wherein the substrates are selected such that the  
second color is substantially blue-violet.

28. The method of claim 27, wherein the substrates are selected such that the  
third color is substantially red-pink.

29. The method of claim 22, wherein the substrates are selected such that the  
colonies of *Aeromonas* and colonies of *Plesiomonas* and *Vibrio* are the third color.

30. A test medium for detecting, quantifying and differentiating general coliforms, *E. coli*, and at least one of the genera *Aeromonas*, *Salmonella* and *Shigella*, said test medium comprising:

a nutrient base medium including ions of a salt;

a first substrate capable of forming a first water insoluble component of a first color in the presence of *E. coli*;

a second substrate capable of forming a second water insoluble component of a second color in the presence of general coliforms; and

a third substrate capable of forming a third water insoluble component of a third color in the presence of one of the genera *Aeromonas*, *Salmonella* and *Shigella*, said third color being distinguishable from said first and said second colors;

all of said colors being visually distinguishable, one of said colors being substantially black.

31. The test medium of claim 30, wherein said first substrate is selected from the group consisting of 8-hydroxyquinoline-  $\beta$ -D-glucuronide, 8-hydroxyquinoline-  $\beta$ -D-galactoside, an esculetin galactoside, an esculetin glucuronide, cyclohexenoesculetin- $\beta$ -D-glucuronide and cyclohexenoesculetin- $\beta$ -D-galactoside.

32. The test medium of claim 30, wherein said first substrate consists essentially of a  $\beta$ -glucuronide.

33. The test medium of claim 32, wherein said second substrate comprises an  $\alpha$ -galactoside and said third substrate comprises a  $\beta$ -galactoside.

34. The test medium of claim 30, wherein said first substrate consists essentially of a  $\beta$ -D-glucuronide which combines with ions of said salt to form a substantially nondiffusible compound.

35. The test medium of claim 30, wherein said third substrate is capable of forming said third water insoluble component of said third color in the presence of general coliforms, whereby general coliforms are indicated in said test medium as a fourth color, said fourth color being a combination of said second and said third colors.

36. The test medium of claim 35, wherein said third substrate is capable of forming said third water insoluble component of said third color in the presence of *Aeromonas*, and said second substrate is capable of forming said second water insoluble component of said second color in the presence of one of *Salmonella* and *Shigella*,

whereby *E. coli* is indicated in said test medium as said first color, the one of *Salmonella* and *Shigella* are indicated by said second color, *Aeromonas* is indicated by said third color, and general coliforms are indicated by said fourth color.

37. The test medium of claim 30, wherein said salt comprises a metallic salt and said first water insoluble component is formed by reaction with said ions, whereby said first color is substantially black.

38. The test medium of claim 30, wherein said first substrate comprises a  $\beta$ -glucuronide, said second substrate comprises an  $\alpha$ -galactoside, and said third substrate comprises a  $\beta$ -galactoside.

39. The test medium of claim 38, wherein said first substrate consists essentially of 8-hydroxyquinoline-  $\beta$ -D-glucuronide, said second substrate consists essentially of 5-bromo-4-chloro-3-indolyl- $\alpha$ -D-galactoside, and said third substrate consists essentially of 6-chloro-3-indolyl- $\beta$ -D-galactoside.

40. A method for detecting, quantifying and differentiating general coliforms and *E. coli* in a test sample, said method comprising the following steps:

providing a nutrient base medium including first and second substrates and ions of a salt;

selecting the substrates such that colonies of *E. coli* produced in the test medium are a first color and colonies of general coliforms produced in the test medium are a second color, the colors being visually distinguishable, one of the colors being substantially black;

inoculating the test medium with the test sample;

incubating the test medium;

examining the test medium for the presence of the colonies of *E. coli* having the first color and the colonies of general coliforms having the second color.

41. The method of claim 40, wherein the salt is a metallic salt, the ions of the salt reacting with one of the substrates to produce the substantially black color.

42. The method of claim 40, further comprising:

including a third substrate in the test medium;

selecting the third substrate such that colonies of *Aeromonas* produced in the test medium are a third color and colonies of *Salmonella* and *Shigella* produced in the test medium are a fourth color; and

examining the test medium for the presence of the colonies of *Aeromonas* having the third color and the colonies of *Salmonella* and *Shigella* having the fourth color.

43. The method of claim 42, further comprising selecting the substrates such that the first color is substantially black, the second color is substantially blue-violet, the third color is substantially red-pink and the fourth color is substantially teal-green.

44. The method of claim 42, further comprising selecting the substrates such that the first color is substantially black, the second color is substantially blue-violet, the third color is substantially teal-green and the fourth color is substantially red-pink.

45. The method of claim 42, further comprising adding an inhibitor so that colonies of one of *Aeromonas* and other *nonenterobacteriaceae spp.* are not produced in the test medium.

46. A test medium for detecting, quantifying, identifying and/or differentiating biological entities under ambient light, said test medium comprising:

a base medium including ions of a salt present in said medium;

a first substrate capable of forming a first water insoluble component of a first color in the presence of a first enzyme and said ions of said salt, said first color being substantially black; and

a second substrate capable of forming a second water insoluble component of a second color in the presence of a second enzyme, said second color being distinguishable from said first color;

whereby aggregations of one type of biological entity producing or containing said first enzyme in said medium are indicated by said first substantially black color and aggregations of a second type and a third type of biological entities producing or containing said second enzyme in said test medium are indicated by said second color.

47. The test medium of claim 46, wherein said first substrate consists essentially of a  $\beta$ -D-glucuronide.

48. The test medium of claim 46, wherein said salt comprises ferric ammonium citrate and said ions comprise ferric ions.

49. The test medium of claim 46, wherein said second substrate consists essentially of a  $\beta$ -D-galactoside.

50. The test medium of claim 46, further comprising a third substrate capable of forming a third water insoluble compound of a third color in the presence of a fourth

5 type of biological entity producing or containing a third enzyme, said third color being distinguishable from said first and said second colors, whereby said test medium is capable of detecting, quantifying, identifying and/or differentiating first, second and third types of biological entities.

51. The test medium of claim 44, wherein said third substrate is capable of forming said third water insoluble component of said third color in the presence of aggregates of the second type of biological entity, but not in the presence of aggregates of the third type of biological entity, so that aggregates of the second type of biological entity appear as a fourth color, said fourth color being a combination of said second and said third colors, aggregates of the third type of biological entity appear as said second color, and aggregates of the fourth type of biological entity appear as said third color.

52. A method of making a test medium for detecting a first type of biological entity and inhibiting a second type of biological entity from growing in the medium, said method comprising:

combining desired substrates with a nutrient base medium;  
adding an inhibitor to the medium; and  
then sterilizing the medium by subjecting the medium to at least 100°C;  
whereby subsequent sterile addition of inhibitor is unnecessary.

53. The method of claim 52, wherein the inhibitor comprises nalidixic acid.

54. The method of claim 52, wherein the second type of biological entity to be inhibited is one of *Aeromonas* and other *nonenterobacteriaceae spp.*